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SILVER-LEAF DISEASE—V.

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By F. T. BROOKS and W. C. MOORE.

I.—INTRODUCTION.

Towards the end of 1922 additional facilities for the continued investigation of Silver-leaf Disease of Fruit Trees were provided by the Development Commission through the Ministry of Agriculture and Fisheries, which have enabled these researches to be prosecuted on a larger scale than hitherto. The increased facilities include a small experimental plantation of plums, kindly loaned for a period of years by Mr. A. R. Fordham, and the provision of a greenhouse suitable for growing plum trees in pots. Certain investigations have also been carried out at the East Malling Research Station and the John Innes Horticultural Institution, by kind permission of Mr. R. G. Hatton and Mr. W. Bateson respectively. Valuable information has been given us from time to time by officials of the Ministry of Agriculture and Fisheries, especially Dr. G. H. Pethybridge, and by others, notably Miss A. D. Mackenzie.

The larger numbers of trees now available for experimental purposes have enabled the researches during the past two or three years to be concentrated chiefly upon the following issues : (1) Can *Stereum purpureum* infect wounds in fruit trees in all months of the year with equal facility ? (2) Does this fungus infect new exposures more readily than wounds of long standing ? (3) What are the best substances for the protection of wounds against this fungus ? These problems are intimately connected, and it is obvious that the right solution of them will have a marked influence upon views as to the time and manner in which operations involving the cutting out of branches of fruit trees should be carried out. The bearing of these problems upon the incidence of Silver-leaf disease is manifest. The sequel will show that considerable progress has been made in the solution of these problems.

Silver-leaf disease is present to a greater or less extent in all the chief plum and apple growing districts of the world. Cunningham (11) has called attention to its prevalence in New Zealand, and Putterill (19) to its existence in South Africa. Güssow (15) reported its occurrence in Canada in 1912, and more recently Heald and Boyle (16) have called attention to the menace of this disease in the important apple-growing districts in the State of Washington. The symptoms and course of the disease in all these countries are essentially the same as in England. It is now universally admitted that the primary cause of the Silver-leaf disease which is associated with the dying back of fruit trees, is the fungus *Stereum purpureum*.

It is not proposed to give a list of new hosts for this disease which have come under our observation during the last three years. Many shrubby plants have

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been seen to be affected by this disease, in which it has not hitherto been recorded. Cotton (10) has recently called attention to the killing of Rhododendrons by *S. purpureum*. In paper IV. of this series Silver-leaf disease in roses was recorded for the first time. Since then other varieties of roses, notably ramblers, have been occasionally seen to be affected by this disease, with the consequent death of the stems and development of *S. purpureum* thereon. A few additional cases of the disease in pears in this country have been observed, but the disease is still extremely rare on this host. In this connection it may be mentioned that Dr. J. Westerdijk informs us that it is easy to produce silvering symptoms in certain varieties of pears in Holland by inoculation with the fungus ; all the inoculations of pear trees which we have made in this country have failed to reproduce the disease.

An indication of the relative suceptibility and resistance of the chief commercial varieties of plums and apples was given in the previous paper of this series. It may now be added that in our experience the comparatively new variety of plum, Purple Egg, is resistant to this disease.

In the previous paper of this series (3) a list of plum stocks was given which had been shown to be affected by the disease upon artificial inoculation with the fungus. It was pointed out that Myrobolan B was the most resistant of those tested : this has been confirmed by another series of experiments. Other stocks which have been shown to be moderately susceptible to the fungus by artificial inoculation (as described in the previous paper) are Mariana, Cluster Damson, St. Julien B, Kroosjespruim, Black Damas C, Myrobolan A, C and D, Bastard Common Mussel, and St. Julien G (Toulouse). Although all these stocks can be artificially infected with Silver-leaf disease, the percentage of recovery amongst them is high, and there is no doubt that all the plum stocks in common use are much more resistant to the disease than varieties of plums such as Victoria and Czar. It is, of course, a matter of common observation that long after the upper part of the tree has been killed by the disease, the stock frequently remains alive and throws up suckers. As pointed out in the previous paper, the influence of the stock on susceptibility of the cultivated variety to the disease is probably indirect ; data are gradually being accumulated in regard to this, but it will obviously be a long time before a confident statement can be made.

In previous papers on this disease (1, 2, 3) attention has been called by one of us to examples of silvered foliage, showing the histological symptoms of the disease caused by *Stereum purpureum*, which was not caused by this or any other fungus, but by a general physiological disturbance, and also examples of silvered foliage in which the " silvering " was due to innumerable punctures of the epidermis by insects. Many similar instances of the first kind have been seen during the last two years, especially in seedling plants and in the

leaves of certain herbaceous plants at the end of the winter. " Silvering " of this kind, however, never has a serious effect upon the plants, and is a comparatively temporary phenomenon. Our present outlook upon these cases of " silvering " is the same as that recorded in previous papers of this series. As regards " silvering " which is not due to attack by *Stereum purpureum* Petri (18)* and Ciferri (8)* have recently made some interesting observations to which brief reference must be made. Petri (18) records the case of a peach tree which threw up silvered shoots after being cut back, in which the appearance of " silvering " was due to an abnormal accumulation of crystals of calcium oxalate in the upper epidermis. He also states that " silvering " in *Viburnum tinus* is sometimes due to the separation of the cuticle from the rest of the outer wall of the epidermal cells. Ciferri (8) records " silvering " in peaches and plums which had been cut back. According to his investigations this was due to the detachment of the cuticle. Both authors agree, however, that the silvering of foliage which is associated with the dying back of branches is entirely due to *Stereum purpureum*.

II. INFECTION EXPERIMENTS WITH SPORES OF *STEREUM PURPUREUM*.

(1) Infection of cut Plum Twigs in the Laboratory.

In " Silver-leaf Disease, IV." (3) it was pointed out that plum twigs could be readily infected in the laboratory with *Stereum purpureum* by placing a spore emulsion on the cut extremities, the other ends of the twigs being kept in water during the experiment. It is now known why it is unnecessary to keep the inoculated extremities of such twigs under moist conditions as we (4) have shown that the spore emulsion is sucked a considerable distance into the vessels, so that the spores and young germ tubes are protected from desiccation during the critical stages of the initiation of infection.

For the last two years experiments of the same kind have been carried out, month by month, to ascertain if infection of cut plum twigs, three to four years old, can be brought about at all times of the year, and to compare the speed of invasion in different months. Both Czar and Victoria twigs have been used in these monthly experiments, but there is no material difference in the reaction to invasion on the part of these varieties, so that the results may be presented together. Uninoculated twigs were always kept as a control, and with the exception of a very small number which became infected with extraneous micro-organisms, these remained healthy until the end of the experiment, showing only a very slight amount of discoloured tissue below the exposed surface.

* We are much indebted to Mr. A. W. Exell of the British Museum (Natural History) for translations of these papers.

From March to August inclusive, infection of these twigs has proceeded rapidly so that in the course of six weeks the fungus has invaded them on an average to a distance varying from four to seven inches. This has been determined both by tracing the discolouration of the wood caused by the fungus and by the examination of sections. Inoculation of cut plum twigs, 9 inches long, in May often resulted in complete permeation by the fungus within six weeks, accompanied by the development of fructifications of *S. purpureum* at about the level of the water in which the twigs stood. From September to February inclusive the rate of penetration has been much less. In September the average invasion was 1 inch, in October $\frac{1}{2}$ inch, and in November $\frac{1}{5}$ inch. The results for December and January are somewhat aberrant, and differ for the two years over which the experiments have extended. In December, 1923, the penetration was 4 inches, but in 1924 it was only $\frac{1}{2}$ inch. In January, 1923, the penetration was $\frac{1}{2}$ inch, and in 1924, $3\frac{1}{2}$ inches, the result in 1924 being comparable with that of December, 1923, i.e., the previous month. In February, 1923, invasion occurred to a distance of 6 inches, and in 1924 to 2 inches. It is clear from these results that, although the initiation of invasion of cut twigs can take place throughout the year, the rate of penetration is much more rapid at one period than another. The difference in rate of invasion during the two periods cannot be wholly due to changes of temperature, for fluctuations of this kind in the laboratory are small. The divergence is probably chiefly caused by physiological differences in the twigs. Although the periods of rapid and slow penetration have been approximately constant during the two years, there is some fluctuation in the rate of invasion during December, January and February. This fluctuation is also probably due to variability in the physiological state of the twigs in the two seasons, for the laboratory conditions of experimentation were as constant as could be arranged.

(2) Infection of cut Laburnum Twigs in the Laboratory.

As is well-known, heart wood becomes clearly differentiated from sap wood at an early stage in the laburnum, the heart wood being brownish-black in colour in contrast to the almost white sap wood. It was thought, therefore, to be of interest to inoculate the cut ends of laburnum twigs with a spore emulsion of *Stereum purpureum* to ascertain if there was any difference in the rate of invasion of heart wood and sap wood respectively. Attention has been previously called in this series of papers to the frequent destruction of laburnum trees by the fungus.

Freshly-cut twigs of laburnum, about ten years old, were placed in water in the laboratory in June, and the newly exposed extremities were covered with a spore emulsion of *Stereum purpureum*. At the same time control twigs were left uninoculated. After six weeks the inoculated twigs showed a marked

difference between the heart wood and the sap wood, the former being covered with *Penicillium* and the latter with rudimentary fructifications of *Stereum purpureum*. Both the heart wood and the sap wood of the control twigs were covered with *Penicillium*. Upon cutting up the inoculated twigs it was found that the sap wood was permeated with the mycelium of *Stereum purpureum* causing the usual brown discolouration, whereas the fungus had not entered the heart wood, it being replaced by a purely superficial growth of *Penicillium*. The growth of *Penicillium* both on the heart wood of the inoculated twigs and over the whole surface of the control twigs was doubtless due to these being kept in a moist atmosphere. Ordinarily, *Penicillium* does not grow upon woody tissues exposed to a dry atmosphere. Attention has previously been called (3) to the fact that *Stereum purpureum* is a fungus which preferably invades woody tissues that contain living cells. The contrast in its behaviour towards the heart wood and sap wood of laburnum is additional confirmation of this view.

(3) Infection of exposed extremities of Plum branches in Nature.

(*a*) *Inoculations made almost immediately after exposure.*

While experiments on the infection of plum twigs in the laboratory were being carried out, similar experiments were made, month by month, in the field.

For this purpose two to three-year-old twigs of Czar plums were cut back and an emulsion of freshly-deposited spores of *Stereum purpureum* was applied two days and four days afterwards. The object of giving two applications of spores was to ensure ample opportunity for infection. About twenty wounds were inoculated each month, of which three or four were cut up after three months and the same number after six months in order to determine by microscopic examination whether infection had occurred. The remainder were left on the tree to see whether they resulted in silvering of the foliage in the immediate neighbourhood of the inoculation. If silvering developed within a reasonable period of time, this also was taken as a sign of positive infection. In the case of the March and April inoculations silvering of the foliage often appeared before some of the twigs were due to be cut up.

The results of two years' inoculations are given in the following table :

	Positive infections.	
	(1923-4)	(1924-5).
April	100%	100%
May	71%	25%
June	0%	0%
July	6%	0%
August	0%	36%
September	—	30%
October	100%	64%
November	21%	50%
December	77%	86%
January	100%	—
February	75%	100%
March	94%	14%

No inoculations were carried out during September, 1923, and January, 1925.

These results indicate that there are striking differences in the chances of infection by *Stereum purpureum* in nature at different times of the year. The most noteworthy feature of these data is the almost complete absence of infection during June and July, and in one year in August, whereas in other months infection takes place sometimes to the extent of 100 per cent. of the inoculations. Exact agreement in the relative amount of infection is the corresponding months of succeeding years is not to be expected because of variations in the weather and other factors, but the results as a whole agree sufficiently to be significant. Slight fluctuations in the periods of maximum and minimum infections from year to year are not surprising.

Most of the twigs inoculated during June, July and August, which did not become infected, were cut up after a suitable interval in order to ascertain the cause of the inhibition of complete infection. Examination showed that the fungus spores had germinated and that the mycelium had penetrated to a distance of about half an inch, but further progress of the fungus had then stopped. The wood permeated by the mycelium was brown in colour as usual owing to the presence of gum in the cells, but, on the margin of the wood affected in this way, there was a narrow zone much more intensely discoloured, and, in fact, almost black. The vessels in this deeply discoloured zone contained even more gum than the region permeated by the mycelium, and the gum was darker in colour than in the latter. The mycelium had not entered this delimiting zone, so that the fungus was completely shut off from the healthy wood beyond. It seems certain that this " gum barrier," as it may be called, definitely prevents the fungus from proceeding further. Thus the lack of infection leading to Silver-leaf disease is not due to failure of initial invasion, but to a pronounced reaction on the part of the host leading to the formation of an impenetrable barrier of gum-like substances. In Silver-leaf disease IV (3) attention was called to the presence of a similar " gum barrier " in Pershore plum trees which had recovered from the disease, and also in pear trees which had been unsuccessfully inoculated with the mycelium of the fungus. It seems therefore that the formation of gum of this kind in the host tissues is generally the cause both of lack of infection and of recovery from infection by this fungus. For reasons which have already been given in the fourth paper of this series it is suggested that the protective gum in the almost blackened zones may be different from the gum of the brown discoloured wood permeated by the mycelium ; it also fills the elements of the wood more completely than does the ordinary type of gum. On the other hand, the deepening of the colour in the " barrier " may be due chiefly to gum being present in greater quantity.

Another proof that protective "gum-barriers" are formed with particular facility during the summer is afforded by the results of a series of experiments in which plum trees in pots in a greenhouse were inoculated during June, 1924 with mycelium of the fungus. Of forty trees inoculated in this way only two ultimately became silvered. Several of the inoculated trees which failed to become silvered were cut up in August, 1925, when it was found that in each of these trees the progress of the fungus had been quickly stopped by the formation of a "gum-barrier." Consequently the trees did not become affected by silver-leaf disease. In previous papers of this series it has been pointed out that it is extremely difficult to bring about successful infection of plum trees by mycelial inoculations during the summer. The reason for this is now apparent in the light of the results just recorded : in all such cases it is the ready formation of a "gum-barrier" which prevents successful invasion.

The reasons for the rapid formation of a "gum-barrier" in June, July and August, thereby preventing infection, are by no means clear. "Gum barriers" are certainly not limited in time of formation to these months, because whenever there is failure to proceed with invasion after initial penetration, and in all cases of recovery from the disease, a "gum-barrier" is responsible for the defeat of the fungus. There is reason to suppose, however, that "gum barriers" are more readily formed in the summer months than at other times of the year. This is probably due to some special physiological condition of the twigs during these months.* Alternatively, these barriers may be the more readily formed then because the fungus perhaps attacks most vigorously during that period. With the enhanced temperatures of the summer the fungus grows faster, although it may be argued that with higher temperatures the protective gum is also more easily formed. If it be true that the protective gum barriers are brought into being by the vigor of attack of the fungus, the ultimate escape from infection can be compared with the well-known case of resistance of certain varieties of wheat to *Puccinia glumarum*, where the ultimate immunity is due to a too vigorous initial attack by the fungus. The almost constant lack of infection during the summer months is, however, probably related to some peculiar feature in the initiation of attack of the fungus during that period, in association with the wound response of the host; otherwise one would expect that invasions by this fungus begun in April would stop in June : that is certainly not the case. It is the early stoppage of invasion in the summer months which is of peculiar interest in these experiments, but whether this is due primarily to the host, or to the fungus, or whether the "honours" are even between the two, for the present remains somewhat

* By the kindness of Mr. T. Swarbrick we have been able to read an account, before publication, of work by him on the blocking of wounds in trees through gum formation. Mr. Swarbrick also finds that the blocking of woody exposures by gum takes place more readily in the summer months than at any other time of the year.

obscure, although the available evidence points to the physiological condition of the host as being most important.

The rate of penetration of plum twigs successfully inoculated with spores of *Stereum purpureum* varies of course at different times of the year; it is doubtless dependant partly upon the temperature and partly upon the concentration of food material available for the fungus in the wood. Invasion during January and December is naturally slow at first but proceeds rapidly as the temperature rises. Dixon and Atkins (13) have shown that there are always some soluble carbohydrates as well as mineral salts present in the tracheæ of deciduous trees, so that food material is available for the fungus throughout the year, altogether apart from the ability of the fungus to secrete enzymes which transform starch and other complex substances. According to the researches of Dixon and Atkins (13) the maximum concentration of soluble carbohydrates in the tracheæ occurs in April, after which there is a considerable fall until a minimum is reached in September. It is of interest that in April, 100 per cent. of the inoculations were successful and that the period of diminishing soluble carbohydrates coincides roughly with the period during which it is almost impossible to bring about natural infection by spores.

There are some striking differences between the results of the laboratory and the field inoculations of plum twigs. Conclusions drawn from the former as to the possibilities of infection in nature would obviously be erroneous. The experiments show that in nature it is almost impossible to bring about infection of fresh exposures during June, July and August, while cut twigs kept in the laboratory are readily infected during these months. There are important differences in physiological state between cut twigs attached to trees, and the divergence in infection is doubtless bound up with these differences.

The results of these inoculation experiments carried out in nature at different times of the year have an important bearing upon the time of cutting out dead wood in silvered fruit trees. By the Silver-leaf Order of 1923, the Ministry of Agriculture insists upon fruit-growers cutting out all dead wood in fruit trees liable to harbour *S. purpureum* before July 15th each year. This date was originally suggested because fresh fructifications of the fungus giving spores are least abundant during the early part of the summer, and it was thought that, if healthy wood were exposed in this process of cutting-out, re-infection would then be least likely to occur. It is also easier to cut out dead wood in fruit trees during the early summer than in the winter, because at the former time there is the clearest differentiation between healthy and dead branches. The results of the above inoculation experiments also demonstrate that it is best to cut out the dead wood in fruit trees in the weeks immediately preceding July 15th, because of the extreme unlikeliness that spores of *S. purpureum* will cause infection during June and July, even if spores of the fungus are then present in

the air. On the other hand, to cut out dead wood in March and April is to court disaster, because in the latter month infection is brought about with great readiness.

(*b*) *Inoculation made after varying periods of exposure.*

In " Silver-leaf Disease, IV." (3) it was suggested that *Stereum purpureum* probably infects newly exposed wood more readily than wood which has been exposed for some time. During the last two years experiments have been carried out to test the truth of this statement. At four different periods in the year plum branches in nature were cut back and subsequently inoculated with a spore emulsion of *Stereum purpureum*, one week, one month, three months, and six months respectively after exposure. On each occasion three branches were treated identically. The following are the results of one of these experiments :

Month of Cutting.	Period of exposure before inoculation.	Number of positive infections out of three inoculations.
January, 1923.	1 week.	3
	1 month.	1
	3 months.	0
	6 months.	0
April, 1923.	1 week.	2
	1 month.	1
	3 months.	0
	6 months.	0
July, 1923.	1 week.	0
	1 month.	0
	3 months.	0
	6 months.	0
October, 1923.	1 week.	0
	1 month.	0
	3 months.	0
	6 months.	0

This table must be interpreted in the light of the results obtained by inoculating plum branches in the field at different times of the year immediately after exposure. Owing to the general absence of infection in July one would scarcely expect infection to take place in the above series of experiments when branches were inoculated three months after exposure in April and six months after exposure in January. Nor on the same basis would one expect infection to occur one week and one month respectively after exposure in July. Otherwise the results confirm the view that *Stereum purpureum* infects woody tissues much more readily at or immediately after exposure than at a later period. Another

series of experiments gave essentially the same results. With regard to the absence of infection after one week's exposure in October, it will be seen from the first table that infection readily occurred during this month when spores were applied almost immediately after the wounds were made.

Woody tissues soon after exposure inevitably become invaded with a variety of micro-organisms, the germs of which are constantly present in the air, so that after an interval of three months there may be a considerable number of invading organisms, which, except in the winter months, will have caused an appreciable amount of discolouration below the exposed surface. It seems clear that a fungus like *Stereum purpureum* with parasitic proclivities cannot pass through tissues occupied by other micro-organisms as easily as through sound tissues. Some of these twigs which did not become infected after exposures of three or six months before inoculation, have been cut up after the lapse of another three months ; for a distance of half an inch to an inch on an average below the surface discoloured tissues are invariably found, which are bounded by a darkly discoloured "gum barrier" similar to that which is characteristic of failure of infection by *Stereum purpureum*. Sections through the invaded tissues frequently showed the characteristic mycelium of *S. purpureum* ; the spores used for inoculation had evidently germinated and had permeated to come extent tissues already invaded by other fungi. The *Stereum* hyphæ could not often be found as far as the special "gum barrier," although other mycelia could be so traced, so it remains uncertain whether the barrier was primarily caused by other invading organisms or by *Stereum purpureum* working in association with them. Invasion of woody tissues by other micro-organisms often leads to the formation of protective "gum barriers," as e.g., after wounds have been left naturally exposed for periods of three to nine months. As stated in "Silver-leaf Disease, IV." (3) there is great diversity of species among the micro-organisms which invade woody tissues to a limited extent, but without acting as dangerous parasites.

(4) Other Infection Experiments.

The results of the main series of inoculation experiments with the spores of *S. purpureum* indicate clearly that the longer the wound has been exposed the more difficult is it for this fungus to infect. After a month's exposure *S. purpureum* rarely infects woody tissues, and no successful invasion by this fungus has been seen where the wood has been exposed as long as three months. It has been shown that with all wounds exposed for a considerable period the extremities become invaded by a variety of micro-organisms. If, for instance, the spores of *S. purpureum* be placed on wood exposed for about a month, invasion by other fungi will have already begun. These other fungi have already altered the nutritive conditions of the wood and they are already established as

competitors with *S. purpureum* when the latter begins to develop. In the majority of cases *S. purpureum* fails to penetrate completely the tissues already occupied by other fungi, and therefore disease does not ensue. In such exposures the spores of *S. purpureum* germinate under moist conditions, but the germ tubes make slower progress than in fresh tissues and soon cease to develop further.

As woody tissues exposed to the air are usually invaded by a considerable mixture of micro-organisms, it was thought desirable to ascertain whether *S. purpureum* could cause invasion through tissues already attacked by specific fungi. For this purpose *Sclerotinia cinerea* and *Diaporthe perniciosa* were chosen as being two of the commonest pathogenic fungi on plum trees.

Cankers of plum branches caused by *Sclerotinia cinerea* were inoculated, both in the field and in the laboratory, with spores of *Stereum purpureum* at a time of the year favourable for infection. None of the cankers in the field experiment were successfully traversed by *S. purpureum*, and only one of those inoculated in the laboratory succumbed to infection, as shown by the fungus *S. purpureum* passing far beyond the limits of the canker. Little emphasis, however, can be laid upon this single success as experience has demonstrated that experiments on cut twigs cannot be used as an accurate guide to behaviour in the field.

In another experiment small plum branches in the experimental plantation were cut back and immediately inoculated with a spore emulsion of *Sclerotinia cinerea*. A month later most of these twigs were inoculated with a spore emulsion of *Stereum purpureum*. A few were cut up to determine the progress made by the *Sclerotinia*, which was only slight. On cutting up the twigs after the lapse of six months it was seen that *S. purpureum* had in no case caused infection.

As far as these experiments go, it seems safe to conclude that under field conditions *Stereum purpureum* does not infect plum trees through tissues already killed by *Sclerotina cinerea*. This conclusion agrees with field observations over many years that plum trees seriously affected by *Sclerotinia cinerea* (Brown Rot) are not more susceptible to Silver-leaf disease than are healthy trees.

With *Diaporthe perniciosa* laboratory experiments only are complete so far, but these also indicate that *Stereum purpureum* is rarely, if ever, able to pass completely through tissues already permeated by the first-named fungus. A considerable field experiment of the same kind is in progress, but the results are not yet available.

In view also of successful infection through leaf-scars (21) and lenticels by other wound parasites attempts have been made to induce infection through these structures by the spores of *S. purpureum*, but entirely without success.

III. WOUND PROTECTION IN FRUIT TREES.

The investigations outlined in " Silver-leaf Disease, IV." (3) on the subject of wound protection in fruit trees have been continued on a large scale during the last two years. In view of the fact that *Stereum purpureum* infects wounds most readily immediately after exposure all the experiments have been carried out on the basis of placing the protective over the wounds directly after these were made. The type of wound generally used for these experiments has been that made by a pruning knife or secateurs in severing branches, but wounds obtained by splitting asunder two branches at a fork have sometimes been employed. Branches two to three years of age have been mostly used for these wound experiments, which have been carried out on plum trees (chiefly Czars) in the experimental plantation. With the more important substances tests have been carried out practically during every month in the year.

Throughout these experiments an emulsion of spores of *Stereum purpureum* has been placed on the protective substance a few days after the latter has been applied, or after a longer interval. The spore emulsion was always added in dry weather, and sometimes more than one application of spores was given. The branches thus treated were generally left in order to see if silvering developed in connection with them, although at certain times of the year they were cut up to determine whether infection had occurred, without waiting until the foliage appeared.

Many different protectives have been used in these experiments, but only the more important of these will be discussed in detail. An important consideration with these protectives is that, for use by fruit-growers, they should be practically harmless to the bark. One of the best of these protectives has had to be discarded because of its toxicity to the tissues.

Antifouling Paint. As pointed out in the previous paper of this series, this paint, which is used on ships' bottoms, gives a splendid surface on drying and does not readily crack, although it is somewhat toxic to the bark. Apart from the latter defect it seems an ideal means of protecting wounds from invasion by *Stereum purpureum* as, notwithstanding innumerable attempts to infect wounds covered by it with this fungus, not a single positive infection has been recorded. Upon request, the manufacturers have made this paint thicker and have reduced considerably its toxicity without altering its advantageous characters, but unfortunately it is still too toxic to the young bark of fruit trees, and therefore cannot be generally recommended for use by fruit growers. The bark of young branches is sometimes killed for two to three inches below the paint. With older bark the toxic influence of the modified antifouling paint is appreciably less, and for covering large wounds in old trees, whether fruit trees or timber trees, its toxicity to the tissues can be ignored.

Red Oxide Paint (as sold). In view of the considerable toxicity of antifouling paint to young tissues other ready-made paints have been used as wound protectives. Red oxide paint (as sold) gives a poorer " surface " than antifouling paint and takes a long time to harden ; moreover, it has a tendency to run down the bark as it is too liquid. The latter defect could, however, be remedied by allowing the paint to thicken before use. This paint (as sold) is less toxic to young bark than is the modified antifouling paint. Below wounds about half an inch in diameter the bark is killed to a maximum of a quarter to half an inch after six months, usually only on that side of the twig towards which the cut slopes and away from the side bearing an attached shoot. This paint is, however, useless for protection against *Stereum purpureum.* In one series of experiments in which a spore emulsion was placed on wounds covered with the paint within a few days of the application of the latter, all of the ten shoots so covered became infected with the fungus.

White Lead Paint (as sold). Ready-made white lead paint gives a better " surface " on wounds in plum trees than does red oxide paint, and it hardens more rapidly than the latter. It shows less tendency to run down the bark than does red oxide paint. It is somewhat less toxic to young bark than red oxide paint, and, when used to cover wounds about half an inch in diameter, the maximum distance to which its toxic influence has extended after six months is one-quarter of an inch, usually only on that side of the twig towards which the cut slopes away from the attached shoot. This paint also is of little use as a protection against *S. purpureum.* In one experiment, eight out of ten wounds covered with the paint and inoculated with a spore emulsion within a few days became infected with the fungus.

Home-made Paints. In view of the difficulties encountered with paints as sold ready for use paints have been made up as follws :

(*a*) *Red Oxide Paint.* To 2 lbs. of red oxide in linseed oil (as bought) add two teaspoonfuls of paste driers and about a tablespoonful of linseed oil. Mix. Then add turpentine gradually and mix well until about $\frac{1}{4}$ pint turpentine has been incorporated.

(*b*) *White Lead Paint.* To 2 lbs. white lead paste (as bought) add 2 teaspoonfuls of paste driers and 2 tablespoonfuls of linseed oil. Mix. Then add 2 tablespoonfuls of turpentine and mix well.

When made in this way these paints have the most suitable consistency for application to tree wounds. Both paints dry quickly and give a good cover which does not crack until a long time after application. The " surface " given by this red oxide paint is nearly as good as that of antifouling paint. Neither of these paints causes greater damage to the bark of young trees than the corresponding paints sold ready for use, and in a considerable number of

wounds protected by them there has been no evidence of toxicity at all. Both of these paints prevent to a very great extent infection by *S. purpureum*. Thus out of fifty wounds treated with the home-made red oxide paint and inoculated with spore emulsions within a few days, only four have become infected. Of forty-four wounds covered with home-made white lead paint and subsequently inoculated only one has become infected. When it is remembered that the spore emulsion used is crowded with spores and that two applications of spores are applied to each of the wounds, these results are very striking. Of the two home-made paints white lead is to be preferred, as it facilitates callus formation and is generally less toxic to the bark than red oxide paint ; on the other hand white lead paint is a little more expensive than the other.

Soft Grafting Wax. This substance is used extensively in nurseries to cover wounds made in propagating fruit trees.* It has been used on a large scale in our experiments. Soft grafting wax is fairly easy to apply to wounds of limited size, is not readily affected by the weather, is not poisonous to the bark, and as it tends to keep the tissues moist, it facilitates the formation of callus.

In one series of experiments with this substance wounds made at different times of the year and immediately covered with it were inoculated a month later with a spore emulsion of *S. purpureum*. None of these became positively infected. After the lapse of a reasonable period these twigs were cut up ; it was invariably found that the wood for a distance of about half an inch to one inch below the surface was discoloured, there being a blackish " gum barrier " on the margin of the discoloured zone. The discoloured wood contained hyphae of various kinds, but the characteristic mycelium of *S. purpureum* was only rarely seen. It is clear that fungi had invaded to a limited extent the tissues below the wax, but had not penetrated sufficiently to be dangerous.

In several other series of experiments carried out in different months the spore emulsion was applied a few days after protecting the freshly cut wounds with grafting wax. Only one wound protected with soft grafting wax has become artificially infected with *S. purpureum* throughout all these series of experiments. In view of this result soft grafting wax can be confidently recommended as a wound protective, especially to cover small wounds to which it can be applied with ease. It is not so easy to cover large wounds with grafting wax and for this purpose one of the home-made paints, which can be applied with a brush, is preferable.

Tar. In the previous paper (3) it was pointed out that Stockholm tar had given very poor results as a wound protective. Further experiments having confirmed this, its use has been discontinued.

* The use of soft grafting wax was recommended to us by Mr. G. N. Bunyard of Maidstone.

At first gas tar gave better results than Stockholm tar, but with greater experience of this substance we have had to modify our opinion of its value as a wound protective. Besides, although gas tar is often innocuous to the bark of plum trees it frequently causes considerable damage to relatively young apple bark. During the last two years many additional experiments have been carried out to ascertain what measure of protection against *S. purpureum* is afforded by gas tar. For this purpose two to three year old twigs of Czar plums have been cut back at monthly intervals and immediately covered with gas tar, a spore emulsion being applied two days and again four days after protection. This set of experiments was carried out at the same time as those to determine the chances of infection of unprotected wounds by *S. purpureum* month by month throughout the year. At most times of the year infection took place as readily through the gas tar as through unprotected wounds, but the results also showed that protection of wounds by gas tar during July and August actually facilitates infection by *S. purpureum*. This is a surprising result, but it has been obtained for two successive seasons, so it cannot be ignored. It will be remembered that infection of unprotected wounds during these summer months is unlikely to occur. Possibly the presence of the tar tends to keep the tissues below moister than in unprotected wounds, and it may be that the influence of the tar tends to prevent the formation of a "gum barrier", which often prevents infection at this time of year.

In another series of experiments wounds of the same kind cut in January, April, July and October were immediately covered with gas tar, and inoculated with a spore emulsion either a week, a month, three months, or six months afterwards. The results for infection through unprotected wounds made at the same time and inoculated in the same way have already been given. Again with wounds covered with gas tar there were as many positive infections as with unprotected tissues. There was also some evidence that the addition of gas tar tended to keep the wounds in a condition susceptible to infection for a longer period than usual. Thus whereas no unprotected wounds became infected after three months' exposure, several of those covered by gas tar for this period did become infected.

When gas tar is applied to a fresh exposure, much of it is sucked a considerable distance into the vessels. However toxic tar in the free state may be to fungi, it is obvious in the light of the above results that when applied to woody tissues its toxicity is not sufficient to prevent *S. purpureum* germinating in association with it and growing through tissues impregnated with it, even when the spores of the fungus are added a few days after the tar has been applied. Upon cutting sections of the wood over which the tar has been placed and spores subsequently added, the hyphæ of the fungus can be seen side by side with drops of tar in the vessels.

In the light of these results one is forced to the conclusion that gas tar, like Stockholm tar, is entirely inefficacious in keeping *S. purpureum* at bay.

Other substances used as Wound Protectives.

Many other substances have been tried as wound protectives, but all of these were soon discarded owing to some defect. Only the briefest mention will be made of these substances.

Collodion. This, dissolved in a mixture of equal parts of alcohol and ether, forms a good film over the surface of wounds, but after a short time the film flakes off and the tissues become exposed. The substance is also difficult to prepare in a suitable form.

Tanglefoot. This and other greasy substances are difficult to spread over wounds.

Water-glass (*cf.* Young (22)). This does not form a good film over wounds, and after about three weeks it dries to such an extent that the wounds appear not to have been covered at all.

Shellac. Shellac dissolved in commercial methylated spirits gives an apparently good surface at first, notwithstanding which infection by *S. purpureum* has taken place through it with some ease. After three months the shellac flakes off in irregular patches.

Asphaltum. This was recommended by Dr. H. Wormald. Dissolved in benzine it gives a good black surface, but this is inclined to break up after about two months. Owing to difficulty experienced in incorporating the right amount of benzine to make a thick paste no very extensive trials with this substance have yet been made.

Sealing Wax. This readily adheres to wounds and does not flake off for a long time, but owing to the necessity of using a flame for its application it was discarded.

Mixture of Potassium Bichromate and Copper Sulphate. Chavestelon (7) claims that a 6 per cent. solution of potassium bichromate mixed with a 6 per cent. solution of copper sulphate is an efficient wound dressing for vines.

The cut ends of plum twigs have been treated with this mixture, but infection by spores of *S. purpureum* was readily induced through it, so it is of little or no value for protection against this fungus.

Rubber Latex. Latex of the Para rubber tree is now available in large quantities in this country, it being kept in a liquid condition by the addition of ammonia. Upon placing it on the cut extremity of a twig it rapidly coagulates, giving what appears to be an impervious coat. More rapid coagulation is, of course, caused by the addition of weak acetic acid, but if this be used, the film

is more irregular than when coagulation takes place naturally. The liquid latex can be easily applied with a brush, but one drawback to its use is that on sloping wounds it is so fluid that most of it runs off the cut. Experiments show that latex is not effective in preventing invasion by *S. purpureum.*

IV. THE FORMATION OF GUM IN PLUM TREES.

As is well-known, plum trees are prone to form large quantities of gum, which frequently exudes in masses from the trunk and branches. Preliminary observations on the formation of gum in the genus *Prunus* have been recorded in " Silver-leaf Disease, IV." (3), in which it is stated that gum-formation is a general pathological phenomenon not always associated with attack by a parasite. Since that paper was written, further work has been done on the mode of gum formation in plum trees. A summary of the investigations on gum formation in woody tissues up to 1922 is given by Czapek (12). Some of the investigators therein referred to consider that the gum comes from the membranes while others hold the view that it is formed from the cell contents.

It is important to distinguish the process of gum formation in meristematic and embryonic tissues from that in lignified tissues. In the former the whole of the cell walls and carbohydrate contents in the cells become converted into masses of gum (gum pockets) as described by Butler (6). In lignified tissues most of the gum, according to our experience, is formed from the carbohydrates in the cells, only a small amount being produced by the gummification of the surfaces of the walls bordering on the lumina of the cells. In the process of gum-formation in lignified tissues the cell walls generally remain essentially intact, quite unlike their complete dissolution in the formation of gum pockets.

The initiation of gum-formation in connection with wounds in plum trees has been followed carefully by examining longitudinal sections of plum wood from a few hours to a few days after exposure in October. After six hours' exposure small yellow blobs appear in the starch grains of the medullary rays just below the surface of the wound ; these stain brown with ammoniated fuchsin and represent the beginning of gum-formation. After two days' exposure the zone nearest the surface contains the yellow, gummy substance both in the ray cells and in the vessels ; below this is a zone in which the gum is present only in the rays ; and below this again no gum is seen. After a month's exposure gum-formation occurs in progressively deeper tissues, the sequence being the same as before. After this interval the outlines of the starch grains originally present in the ray and wood parenchyma cells near the surface are obliterated and no reaction for starch is obtained upon staining with iodine, the starch having been completely replaced by irregular masses of gum. The gum formed in the vessels during the interval is chiefly derived from soluble carbohydrates already

therein or from those which diffuse into them from the ray cells after hydrolysis of the starch. During October, when these experiments were carried out, it must be remembered that the rate of discoloration of the tissues below the exposure is comparatively slow. During the summer the sequence of events is more rapid. In the early stages of the formation of a " gum-barrier " the medullary rays in the immediate vicinity lose most of their starchy contents by hydrolysis, most of the soluble carbohydrates passing into the vessels, to be deposited as dense masses of gum. In a " gum-barrier " comparatively little gum is laid down in the medullary ray cells themselves. It has already been pointed out that the " gum-barrier " is laid down just beyond the limits reached by the mycelium. If a wound be inoculated with a spore emulsion of *S. purpureum* immediately after exposure, the course of events in respect to gum formation is the same except that gum is formed more copiously and more rapidly once the initiation of infection has begun.

It is interesting to note in this connection that Dorsey and Strausbaugh (14), investigating winter injury to plum trees in N. America, found that the storage materials of the wood were in part transformed into gums and tannins by injury of this kind.*

Coster (9) has recently investigated the formation of gum in woody tissues of the teak tree in response to injury. He maintains that the gum-like substances are formed indirectly from starch. This is essentially the view put forward by Brooks and Bartlett (5) and by others, which has been advocated in earlier papers of this series. Coster (9) also concludes that in teak the ray cells must first be dead before the starch can be changed into gum, the wound reaction of living cells being to form tyloses. In plum wood it is very doubtful whether the ray cells are dead at the times of the initiation of gum-formation, which, as pointed out above, begins within six hours of wounding. Gum formation in connection with fungal invasion proceeds in advance of the mycelium, and again it is doubtful whether in the early stages of this process the ray cells are dead. One important difference between teak and plum is that in the latter tyloses are practically never formed, the universal response in the plum to pathological conditions of whatsoever kind being the formation of gum.

V. NATURAL RECOVERY OF SILVERED FRUIT TREES.

Attention was called in " Silver-leaf Disease, IV." to the frequent recovery of susceptible varieties of plum from the disease in certain seasons. It was pointed out that this recovery was due to the progress of the fungus in the tissues having been impeded by the formation of a " gum-barrier," with the result that in time the fungus died. This interpretation of the immediate cause of recovery has been abundantly confirmed during the past two years.

* We are indebted to Prof. J. H. Priestley for calling attention to this paper.

It is only trees of a certain vigour of growth that recover in this way without treatment, and it is reasonable to suppose that only such trees can effectively form the "gum-barriers" which bring about the defeat of the fungus.

Recovery from the disease is more pronounced after a dry season, doubtless owing to the more ready formation of "gum-barriers" then. Why this thould be so is not yet known, and an explanation of the phenomenon is not likely to be forthcoming until there is a much fuller knowledge of the physiology of woody plants than exists at present.

It can at any rate be confidently stated that the chances of recovery from silver-leaf disease depend chiefly upon the vigour of the tree. Affected trees growing in poor soil or lacking in root development practically never recover, and sooner or later are killed by the disease.

There is no doubt that the amount of recovery from this disease is considerably greater than was formerly believed to occur, even with susceptible varieties of plums such as Victoria and Czar. One group of Czar trees (about fifteen years old) in grass which has been under observation for the last three years has given the following results :—

		Number healthy, 1925.	Percentage recovery.
Slightly Silvered, 1922 ..	19	13	68
Moderately Silvered, 1922 ..	23	15	65
Heavily Silvered, 1922 ..	11	0	0
Total..	53	28	53

Thus more than half the trees which were slightly or moderately silvered in 1922 are now healthy. These trees have received no special treatment and have not been manured in any way. It will be noted that none of the trees originally badly affected by the disease have completely regained their health ; this has been noted on other occasions also, and there seems to be a certain degree of attack beyond which it is almost hopeless to expect recovery.

Another way of attempting to evaluate the amount of recovery in this group of trees is as follows :—

Marks were assigned to the trees according to the following scale :

Healthy	0	
Traces of silvering	1	(None of the trees showed traces only in 1922),
Slight silvering	2	
Moderate silvering	3	
Heavy silvering	4	
Killed by the disease	5	

From a comparison of the marks assigned in 1925 with those in 1922 the percentage recovery can be calculated. The recovery amounts to 54 per cent., which again is very considerable for the whole group of fifty-three trees.

As a contrast to the result just outlined the behaviour of another group of Czar trees of about the same age, growing within a mile under conditions of clean cultivation, but much less vigorous, may be mentioned. Here of ninety-three trees which were moderately silvered, only one recovered from the disease, twenty-four remained silvered to approximately the same extent, and no less than sixty-eight died within a period of two or three years.

The vigour of the tree is therefore a potent factor in determining whether silver-leaf disease is to have a fatal issue or not.

Another illustration of the potency of natural recovery where trees are growing vigorously may be given from the East Malling Research Station records by courtesy of Mr. R. G. Hatton :—

	Trees moderately silvered in 1922.	Healthy in 1925.
Pershore	18	14
Victoria	22	10
Giant Prune	8	6
Czar	22	19
Purple Egg	7	7
Total	77	56

These trees, which were planted as maidens in 1921, were not treated in any special way. It will be seen that more than half of them have entirely recovered from the disease.

VI. EXPERIMENTS ON THE AMELIORATION OF SILVER-LEAF DISEASE.

(1) Experiments with Manures.

In view of the considerable amount of natural recovery from this disease it might be expected that the recovery-rate might be increased by appropriate manurial treatment of silvered trees through causing enhanced vigour. At present little information is available as to the response of fruit trees to manurial treatment, and in the absence of such guidance some of the commoner substances used in agricultural practice have been employed.

In one experiment at Melbourne a group of 116 moderately or heavily silvered Czar plum trees, about fifteen years old, and growing in grass, have been under continued observation and treatment during the past three years. These trees were on good land (loam overlying chalk) and were of fairly vigorous growth, as

shown by the abundance of new shoots put forth from time to time. Groups of ten of these trees were treated with kainit, basic slag, or sulphate of ammonia, either separately or in combination, at the rate of 4 lbs. each per tree, two applications being given in December and March respectively in each of the years 1922-3, 1923-4, and 1924-5. At the same time forty-six of these silvered trees were retained as controls without treatment. For the purpose of evaluating results points were assigned to the trees according to the scale previously mentioned, viz. :

Healthy	0
Trace of silvering	1
Slight silvering	2
Moderate silvering	3
Heavy silvering	4
Dead	5

Comparisons were made year by year, and the percentage amount of recovery calculated.

The following are the results after treatment for two and three years respectively :

No. of Trees.	Treatment.	No. of trees which have totally recovered since 1922.		Percentage recovery on point system.	
		1924.	1925.	1924.	1925.
10	Kainit, Basic Slag, Sulphate of Ammonia	3	7	45	76
10	Kainit, Basic Slag	4	7	63	85
10	Kainit	4	6	49	58
10	Kainit, Sulphate of Ammonia..	2	5	22	44
10	Basic Slag, Sulphate of Ammonia	5	6	73	61
10	Basic Slag	3	5	44	59
10	Sulphate of Ammonia ..	3	3	27	21
46	No Treatment (CONTROL) ..	14	18	44	51

The noticeable feature of these results is the enhanced recovery shown in 1925 by trees treated with kainit + basic slag + sulphate of ammonia, and with kainit + basic slag. On the other hand, treatment with sulphate of ammonia appears to have depressed the rate of recovery, and neither kainit nor basic slag alone appears to have had appreciable effect. It must be remembered that heavier applications of these manures have been given than would be applied in ordinary commercial practice, and that no less than six applications of each manure have been given. Even the benefit apparently accruing from treatment by kainit and basic slag in combination is of doubtful commercial value in view

of the cost involved. The experiment has, however, been continued for three years to show what is the probable value of manuring silvered, but fairly vigorous fruit trees under critical conditions of observation.

As a contrast to the above experiment silvered plum trees at Melbourne, weak in growth and situated on poorer land in clean cultivation, were manured in various ways. The trees were chiefly Czars, but a few Monarchs were included. They were of about the same age as the trees referred to in the previous experiment. Two applications of each kind of manure were given in December and March in each of the years 1923-4 and 1924-5. The same amounts of artificials were given, and with farmyard manure liberal dressings were applied. In view of the large number of silvered trees which died in the course of this experiment, it is not worth while to attempt to evaluate the results on the point system.

The results were as follows :

(1) *Treatment with kainit.*

16 trees were treated ; of these 10 were moderately, and 6 heavily silvered at the commencement of the experiment. In September, 1925, the condition was :

9 dead (8 died in 1924).
4 heavily silvered.
2 moderately silvered.
1 slightly silvered.

(2) *Treatment with basic slag.*

16 trees were treated ; of these 4 were slightly, 8 moderately, and 4 heavily silvered at the commencement of the experiment. In September, 1925, the condition was :

11 dead (10 died in 1924).
4 heavily silvered.
1 moderately silvered.

(3) *Treatment with kainit + basic slag.*

15 trees were treated ; of these 4 were slightly, 6 moderately, and 5 heavily silvered at the commencement of the experiment. In September, 1925, the condition was :

10 dead (8 died in 1924).
2 heavily silvered.
2 slightly silvered.
1 healthy.

(4) *Treatment with sulphate of ammonia.*

18 trees were treated; of these 6 were slightly, 6 moderately, and 6 heavily silvered at the commencement of the experiment. In September, 1925, the condition was:

9 dead (all dead in 1924).
1 heavily silvered.
4 moderately silvered.
1 slightly silvered.
3 healthy.

(5) *Treatment with farmyard manure.*

18 trees were treated, twice in 1923-24, and once in 1924-5; of these 4 were slightly, 10 moderately, and 4 heavily silvered at the commencement of the experiment. In September, 1925, the condition was:

9 dead (7 died in 1924).
1 heavily silvered.
4 moderately silvered.
2 slightly silvered.
2 healthy.

(6) *Control.*

14 trees, all of which were moderately silvered at the commencement of the experiment. In September, 1925, the condition was:

5 dead.
6 heavily silvered.
2 slightly silvered.
1 healthy.

These results indicate that it is useless to manure silvered fruit trees the growth of which is poor. These trees, including those in the control, have deteriorated rapidly, and afford a striking illustration of the damage which can be done by this disease to plums of weakly growth.

Another experiment on the effect of manurial treatment of silvered plum trees has been carried out in Worcestershire by Mr. G. C. Maltby, with the co-operation of one of the writers. The trees were very large Victorias, about fifty years old, and still fairly vigorous notwithstanding their age. They were growing on a heavy loam overlying lias. During the winters of 1923-4 and 1924-5 the trees were treated twice, in December and April, with kainit or basic slag or with a combination of these, at the rate of 8 lbs. of each per tree per application. Marks have been assigned to these trees on essentially the same

basis as in the Melbourne experiment on trees in grass, and the percentage recovery or deterioration calculated. The results are :

No. of Trees.	Treatment.	No. of Trees which have totally recovered since 1923.		Percentage recovery (+) or deterioration (—) on the point system.	
		1924.	1925.	1924.	1925.
12	Kainit	3	5	+29	+13
12	Basic Slag	6	6	+58	+47
12	Kainit and Basic Slag	1	1	—21	—39
17 (i.e. 8 + 9)	Control (Plots B. and E. together)	4	5	+ 6	+ 8
8	Control (Plot B.)	4	5	+47	+63
9	Control (Plot E.)	0	0	—21	—24

In this experiment basic slag is associated with the best results, but, in contrast with the experiment on trees in grass at Melbourne, treatment with a combination of kainit and basic slag has been followed by an increase in the disease. The difference in behaviour of the two control plots (B and E), however, shows how hazardous it is to draw conclusions from the behaviour of small numbers of trees.

In all the manurial experiments outlined above it must be remembered that the number of trees receiving any one specific treatment is small, and that, in consequence, the experimental error is large. The difficulty, however, in getting larger numbers of trees to work with is very great and probably insuperable.

To sum up the results of these manurial trials so far, one may say that with silvered trees of average vigour there is some chance that treatment with basic slag will assist recovery, and to a lesser extent this is true of kainit. On other classes of soils the results may be different. What is wanted is treatment that will produce the maximum vigour of growth, for it is under such conditions that the tree tends most to recover from the disease. The fruitgrower will usually be the best judge of what is most likely to be efficacious in this respect. On the other hand, with silvered trees that are weakly in growth it is useless to treat them manurially in the hope of facilitating recovery.

Claims are sometimes made that certain proprietary substances when applied to the soil will cure silver-leaf disease. Those who make these claims frequently ignore the fact that fruit trees not uncommonly recover from the disease without treatment. Some of these claims have been investigated on a large scale with an adequate control, and although these trials are not yet complete, one can state confidently that, as far as the investigations have proceeded, none of these claims have been substantiated.

(2) Experiments with Ring Barking.

It is sometimes claimed that silver-leaf disease can be cured by cutting away a narrow ring of bark two-thirds or three-quarters around the circumference of the silvered branch or main stem if the tree be generally silvered, the ring being made a short distance below the level which, it is supposed, the fungus has reached. We have seen small numbers of trees which have been treated in this way and have subsequently recovered, but in these cases there has been no adequate control experiment.

Large-scale experiments are now in progress to try to determine whether ring-barking can be looked upon as a means of cure or of facilitating recovery. One effect of ring-barking is to cause a disturbance in the normal flow of food substances in the wood and bark in the vicinity of the ring, which may indirectly impede the progress of the fungus. Again, in ring-barking, profuse quantities of gum exude from the ring as a wound response and are also found in the adjacent tissues. These masses of gum which collect in the wood may perhaps mechanically prevent the progress of the fungus as in the formation of "gum-barriers" referred to in the earlier part of this paper, and thereby bring about the defeat of the parasite. Those who advocate ring-barking as a means of control suggest that the gum which accumulates in the ring should be removed from time to time. This was done in our experiments.

The experiments which have been made in this connection are not all complete, but it is desirable to give the results which have accrued to date.

In the first experiment the trees were chiefly Czar plums with a few Monarchs about fifteen years old, growing rather weakly in clean cultivation. Sixty-eight of these trees, all of which were moderately or heavily silvered, were selected in 1922. In June, 1923, eighteen of these were ringed once either towards the base of the silvered branch or on the main stem at a level below that to which it was believed the fungus had reached, the ring being one-quarter to half-an-inch wide. Twenty-eight others were ring barked twice on the same date, with an interval of about six inches between the two rings, the intact portions of bark in the rings being on opposite sides of the branch or main system. The remaining twenty-two trees were left as a control.

The condition of the trees in September, 1925, was as follows :—

Trees ringed once (18).

10 dead.
5 heavily silvered.
1 moderately silvered.
1 slightly silvered.
1 healthy.

Trees ringed twice (28).

20 dead.
3 heavily silvered.
1 moderately silvered.
1 slightly silvered.
3 healthy.

Control (22).

14 dead.
4 heavily silvered.
1 moderately silvered.
1 slightly silvered.
2 healthy.

It is clear that in this experiment, in which the trees were of rather weak growth, ring-barking has not helped to stay the progress of the disease.

In another large-scale experiment the trees (Victoria plums) were ring-barked soon after the trees were inoculated with *Stereum purpureum*, to ascertain whether the ringing would have any effect in preventing the development of the disease. These Victorias were about eight years old, and were growing vigorously. Fifty-four of these trees were inoculated in December, 1924, and thirty-seven of them were ring-barked either once or twice below the place of inoculation soon afterwards, the remaining seventeen being inoculated, but not ring-barked.

The results in September, 1925, were :—

Trees ringed once (19).

1 heavily silvered.
10 moderately silvered.
5 slightly silvered.
3 healthy.

Trees ringed twice (18).

12 moderately silvered.
5 slightly silvered.
1 healthy.

Control (17).

10 moderately silvered.
6 slightly silvered.
1 healthy.

It is too early to draw conclusions from this experiment, although it is clear that ringing does not prevent the development of the disease. As far as the

experiment has gone, there is no difference in the reactions of the three groups of trees.

Until the result of this experiment on vigorous trees is complete, we must retain an open mind on the efficiency of ring-barking, although as far as our experiments have yet gone, we see no reason to recommend this practice as a means of alleviating silver-leaf disease.

A considerable experiment also on these lines was carried out at East Malling Research Station in 1923 in co-operation with Mr. R. G. Hatton. Forty-eight plum trees of different varieties, which were silvered in 1922, were ring-barked in April, 1923, forty-eight trees equally silvered being kept as a control. All these trees were growing well. The results in 1925 were :—

	No. of Trees Ringed.	Complete recoveries in 1925.	No. of Control Trees.	Complete recoveries in 1925.
Pershore	16	11	16	11
Victoria	17	3	17	3
Giant Prune	4	4	4	3
Czar	6	3	6	3
Purple Egg	5	5	5	5
	48	26	48	25

There is no significant difference in the recoveries in the two sets of trees.

VII. TREATMENT.

It has long been advocated in this series of papers that one of the most important measures to take in regard to silver-leaf disease is to prevent the fungus from fructifying within and on the confines of fruit plantations. This attempt to abolish the more direct sources of infection in accordance with the principles of plant sanitation has now been enforced for some years by administrative order of the Ministry of Agriculture and Fisheries, with the result that in the chief fruit-growing districts of the country thousands of fruit trees, killed by the disease, have been removed from being a menace to healthy trees in the vicinity. It is more difficult to ensure the destruction of the fungus on the stumps of trees, particularly birch, poplar and willow, just outside the limits of fruit plantations, but every effort should be made to do this. It is, of course, impossible to destroy completely such a ubiquitous fungus as *S. purpureum*, but we are convinced that the action taken by the Ministry of Agriculture and Fisheries in recent years has been the means of checking to some extent the alarming increase of the disease which was prevalent a few years ago.

The Silver-leaf Order of 1923 insists that dead wood in fruit plantations liable to harbour *S. purpureum* should be cut out by July 15th. The cutting out of dead wood in the early summer was formerly suggested both because it is more easy to see then the difference between dead and living branches, and because at that time of the year the fruiting bodies of *S. purpureum* are least abundant in a sporing condition. Our work of the last two years has also shown clearly that in the months of June, July and to a lesser extent, August, it is almost impossible for *S. purpureum* to cause infection of a fresh wound. For this reason also the cutting out of dead wood—for this often results in the exposure of healthy tissues, should be carried out in the early part of the summer. It is best to cut out the dead wood from otherwise living trees in June rather than July, because this gives a period of about two months before the fungus is again readily able to cause infection, and because it has been shown that in any case a wound exposed for more than a month is but rarely invaded by *S. purpureum.* From every standpoint therefore the cutting out of dead wood in living fruit trees should be done in June rather than at any other time of the year. If the work cannot be completed then, it should be finished as early as possible in July. The same argument of course applies to the thinning out of branches in healthy trees. Hitherto most of the thinning and pruning of fruit trees has been done in the winter, but there is now ample evidence that from the standpoint of plant pathology this is fundamentally wrong. These operations have been performed in the winter because other work is less pressing then, but the point of view on this connection should be revised in the light of the results recorded in this paper. From the standpoint of pressure of work fruitgrowers will probably find it easier to cut out dead wood in June rather than July. On the other hand, it is dangerous to cut out dead wood and other branches in May, as during that month the fungus often readily causes infection.

In connection with the cutting out of dead wood in fruit plantations, it may be mentioned that the writers have never seen fructifications of *S. purpureum* developing upon branches of plum trees killed by *Sclerotinia cinerea, Diaporthe perniciosa,* or *Cytospora sp.* It is, however, good husbandry to cut out these dead branches, whether they are liable to produce fructifications of *Stereum purpureum* or not.

The advisability of cutting out silvered branches of fruit trees depends chiefly upon their condition and upon the skill with which the operation is effected. If the trees are growing well, it is usually best not to cut out the silvered branches unless these have begun to die back, for, as has been shown in the earlier part of this paper, there is a reasonable chance of complete recovery in the course of time. With apple trees the chances of recovery are particularly good. We are aware, however, that some fruitgrowers, who take particular care of their plantations, have controlled the disease successfully by cutting out

in the early summer (June and July) branches of plum trees in the initial stages of silvering. Where branches are actually dying back they should of course be cut out in June and early July wherever possible. A few branches or occasionally entire trees may die from this disease between mid-July and the autumn. In such cases the dead wood should be destroyed without delay to prevent the fungus from fructifying.

Plum branches frequently break when they crop heavily, and the branches are sometimes broken by careless gathering of the fruit. Such wounds are a prolific source of infection of silver-leaf disease unless attended to. Branches which have been broken should be cut back flush with a larger branch or with the main stem immediately after the fruit has been gathered. In some plantations of heavy cropping varieties of plums like Victoria or Czar, it is now the practice to support the over-laden branches in some way or other, and this is the means of preventing a good deal of breakage.

Wounds should, of course, be protected either with soft grafting wax or with one of the paints described in the earlier part of this paper. In view of the results of our experiments gas tar as well as Stockholm tar should no longer be used as a wound protective, as it has been found entirely ineffective in preventing infection by *S. purpureum*, If a wound protective is to be used successfully it must be applied immediately after the wound is made, as that is by far the most susceptible time for invasion by *S. purpureum*. In nurseries particularly it is of the utmost importance to protect wounds made in propagation.

Anything that will stimulate the growth of silvered trees will be advantageous in facilitating recovery from the disease. Good cultivation and effective drainage are essential if the trees are to have a reasonable chance of throwing off the disease. On some soils the addition of a phosphatic or potash manure may be advantageous in increasing vigour.

We have also seen many fairly badly silvered trees recover after the following treatment. The most heavily silvered branches have been cut out in the early summer with a view to diverting the available food to a smaller number of slightly silvered or healthy branches. The result has been so to increase the vigour of the branches which remain that the tree has completely recovered and has, so to speak, been built up anew. In this connection it should be pointed out that badly silvered trees frequently give rise to new shoots immediately below the junction of branches and trunk, which assist in this process of the re-creation of the tree. This is not an operation which can be universally recommended, because great care is needed in the selection of the branches to be retained for the purpose of rebuilding the trees, but, for a fruitgrower who has the time and inclination to work upon his trees as individual units, this procedure can be recommended.

It is particularly important in plum growing to select soils favourable for growth, and trees which have been worked upon known stocks, preferably Pershore, Brussels, Brompton, Myrobolan B., or Common Plum by reputable nurserymen. Then if silver-leaf disease intervenes during the subsequent development of the plantation, the trees will probably possess sufficient vigour of growth to throw off the disease to a great extent. With the large extension of fruit-growing in this country during the last thirty years plums have not infrequently been planted in unsuitable soils with the result that when attacked by silver-leaf disease they have succumbed without a struggle.

VIII. EXPERIMENTS ON THE TOXIC INFLUENCE OF *STEREUM PURPUREUM* ON THE SHOOTS OF TREES.

It is well-known that the manifestations of silver-leaf disease in fruit trees are frequently exhibited above the region of the woody axes invaded by the fungus, and, as pointed out in the fourth paper of this series (3), signs of the disease occasionally appear below the region permeated by the fungus. On the other hand *S. purpureum* sometimes kills trees, e.g., beech and birch, without there being any preliminary symptoms of silvering of the foliage (1, 2), and as recently described by Cotton (10) in Rhododendron.

The usual silvering of the foliage on twigs unoccupied by the fungus is one of the best-known examples in plant pathology of action at a distance. These symptoms are usually explained by supposing that the fungus in its growth in the woody parts secretes a substance which, upon being carried to the leaves in the transpiration current, causes, either directly or indirectly, the phenomenon of silvering owing to the separation of the cells one from the other through the dissolution of the middle lamella. Tutin (20) has shown the pectin content of silvered leaves is less than in healthy leaves. Microscopic examination of a recently expanded silvered leaf shows that the chloroplasts are normal, the alteration in appearance of the leaf being a purely optical effect. At a later stage, however, many silvered leaves show a distinct yellowing of parts of the lamina. This may be followed by the browning of parts of the yellowed areas or of the whole leaf in the case of severe attack. The yellowing parts of a silvered leaf contain chloroplasts undergoing disorganisation and the brown areas denote regions of dead cells. It is evident therefore that the influence of the fungus on the leaves is a complex one, involving several different types of changes.

Mayo (17) in investigating the enzymes of *Stereum purpureum*, has shown that although a pectosinase is present, it is produced in relatively small amount. It is probable that the dissolution of the middle lamella of the leaf cells, which leads to silvering, is not caused directly by the pectosinase secreted by the

fungus, but through the liberation of pectosinase in the cells themselves, stimulated by some other substance or substances carried to the leaves by the activity of the fungus below. The same substances at a later stage and in greater concentration may also indirectly produce the other pathological effects evident in the leaves.

On the assumption that substances are secreted by the fungus in the woody tissues which, upon being carried up in the transpiration current, produce these pathological effects in the leaves, it might be supposed that an extract of the fungus, or, alternatively the culture fluid in which the fungus has been growing, would produce similar effects upon the foliage of a plum tree when injected into the woody parts, or upon the foliage of twigs when these were placed in one or other of these fluids. Many experiments have been carried out in this connection.

In one series of experiments the fluid used was an asparagin-glucose-mineral salt medium in which the fungus has been growing about two months, filtered through a Buchner bougie. Plum twigs with buds just beginning to expand were placed in the fluid, which was renewed at frequent intervals and which was kept from contamination as much as possible. Similar twigs were kept in distilled water. Preliminary experiments had shown that it was necessary to cut off daily about half-an-inch of the part of the twigs immersed in the fluid in consequence of the rapid formation of gummy substance at the absorbing extremity, which prevented the free uptake of the fluid, and which alone might cause wilting. The control twigs were treated in the same way, although the formation of gum at the cut extremities of these was much less. Within a week the expanding leaves of the twigs in the filtered culture fluid showed brown patches around the margin, especially at the tip, and were beginning to wilt, whereas the leaves of the control twigs were entirely unaffected. After ten days all the leaves of the twigs in the culture fluid had wilted and the discolouration was more pronounced, whereas the leaves of the control twigs were still normal. It seems clear therefore that the filtered extract of the fluid in which the fungus had been growing exercised a toxic influence on the leaves of the twigs immersed in it, although no silvering was apparent. The toxic influence here appeared similar to the last phases of silvered leaves in nature in which brown, necrotic areas become apparent.

In another experiment which was carried out when Mr. H. H. Storey was associated with these investigations, healthy plum leaves were placed in (*a*) distilled water, (*b*) boiled plum wood extract, (*c*) the filtered fluid from a culture of the fungus on plum wood. Three leaves were placed in each liquid. After two days all the leaves in the culture fluid were brownish yellow, one leaf in the boiled plum wood extract was somewhat yellowish, the other two being normal, and the leaves kept in distilled water were healthy. The result of this experiment

also points to a toxic influence exercised by the fluid in which the fungus had grown.

In June, 1923, four plum trees in pots were injected with the culture fluid in which the fungus had been growing for four months, the fluid being filtered through a Berkefeld bougie. For the injection a hole was bored into the main stem of the tree, just above soil level, and there was inserted into the hole a glass tube that led upwards to a reservoir in which the fluid was placed. The glass tube was sealed into the stem with plasticine. Control trees were injected with distilled water at the same time. Under these conditions the fluid was readily taken into the stem.* The first effects were seen after four days, when leaves on the same side of the tree as the injection began to turn brown at the margins and wilt, whereas the leaves of the control trees were normal. The inflow of the culture fluid ceased after six days, by which time many leaves on the side of the injection were wilted and brown. The cessation of intake was due to the formation of gum at the place of insertion of the glass tube. Here again there was definite evidence of a toxic influence on the part of the culture fluid, but the symptoms of silvering were entirely absent.

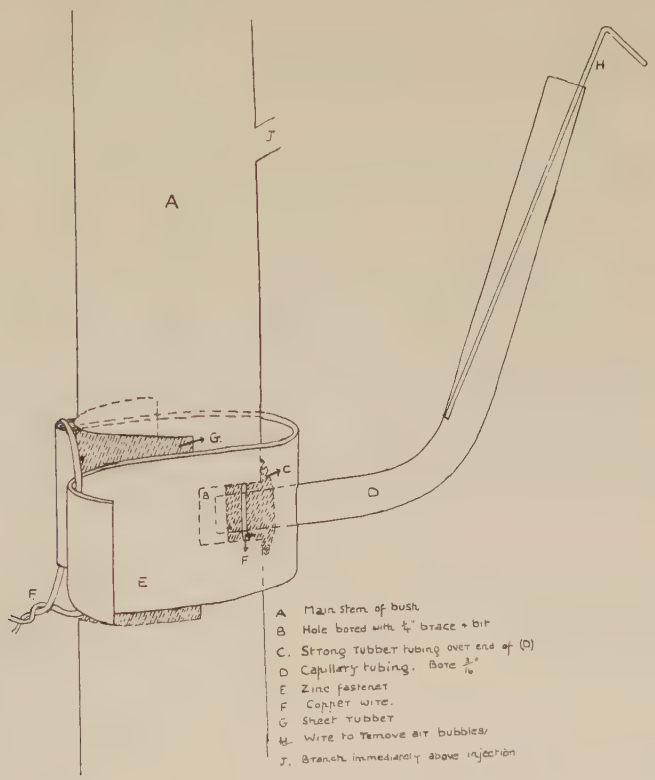
It was thought that perhaps the culture fluid was being used at too great strength so, after filtering, it was diluted to a quarter of the normal concentration and used for the injection of other trees. In this case no positive effect was observed.

In the summer of 1924 other experiments were carried out upon plum trees in a greenhouse. Under these conditions there was great difficulty in keeping the plasticine intact. Another drawback was that, owing to the large size of the reservoir, the culture fluid, notwithstanding careful filtering, became rapidly contaminated. Various modifications of the injection apparatus were tried, and finally one which was kindly suggested by Dr. G. H. Pethybridge was found entirely satisfactory. An annotated diagram of this apparatus (natural size) is reproduced on page 93.

Six plum trees were injected in this way, the place of injection being a short distance below a side branch. Owing to the smallness of the reservoir the filtered culture fluid had to be replenished frequently, but this had the advantage that bacterial contamination was considerably reduced. In five out of the six trees several leaves on the twigs immediately above the place of injection wilted and turned brown within six days of the beginning of the experiment, and other leaves which did not wilt showed within a few days a pronounced yellowish mottling. After fifteen days the intake stopped. It seems that here was evident the yellowing effect which is often apparent in silvered leaves before the development of the brown, necrotic areas.

* The readiness with which fluids are taken up by injection has been utilised by C. B. Lipman and A. Gordon. ("Further Studies on New Methods in the Physiology and Pathology of Plants," Journ. Gen. Physiol., p. 615, 1925.)

In 1925 modified experiments were carried out with the same apparatus, but this time the injections were commenced in March, when the buds of the trees in the greenhouse were just on the point of opening. In these experiments it was thought that the influence of the deleterious substances in the culture fluid might be more evident if they came into contact with the very young developing leaves, for it is known that the symptoms of silvering are best developed when the young leaves become silvered while within the bud. A difficulty in previous



experiments was the cessation of intake after about ten days, owing to the formation of gum at the wounded surface. This was overcome by making a series of injections one above another, at distances apart of about two inches, at weekly intervals, so that the opening buds in the upper region of the main axis were constantly under the influence of the injected fluid. As the tubes were removed from the lower holes the latter were filled with sealing wax.

In these experiments the culture fluid in which the fungus had been growing was filtered through ordinary filter paper as experience had shown that bacterial contamination could not be entirely avoided. Some of the fluid, however, was sterilised after filtration by boiling. Both the unboiled and the boiled fluid were

used in three concentrations : (1) normal, (2) one-fifth normal, (3) one-twentieth normal. Several trees were injected with each of these fluids, both in the greenhouse and on older trees in the open.

At the same time control experiments were carried out by using the sterile culture fluid which had not been inoculated with the fungus, and also by boring holes at intervals upwards in the stems of comparable trees to see if the partial interruption of the transpiration current had any effect.

All the injections with full strength culture fluid in which the fungus had grown, whether boiled or not, caused wilting and browning of the expanding leaves directly above the places of injection, with the same effect, but less marked, on buds in the near vicinity of the line of injection holes. In many cases too some of the buds immediately over the places of injection were killed outright. With the same fluids diluted to one in five and one in twenty, the same effect was still evident, except in the case of those which had been boiled. The effect of the injection of the fluid diluted to one in twenty was less marked than with that of greater concentration. In the control experiments no adverse effect was observed. These particular experiments lasted for six weeks. Although the more serious toxic effects were evident, there was no sign of silvering. If a young leaf did not become affected in the manner described at an early stage, it expanded normally and assumed a healthy green colour. In view of the adverse effect produced by the full strength culture fluid after boiling it would appear that the toxic substance was in part at any rate thermo-stable.

IX. SUMMARY.

1. Additional field observations upon the incidence of Silver-leaf disease are recorded. Attention is called to a slight increase of the disease upon roses, especially ramblers.

2. The ability of spores of *Stereum purpureum* to infect freshly exposed wood tissues of fruit trees has been tested throughout the year. It has been found that the fungus can generally infect fresh wounds readily throughout the year except during June, July, and August. The causes of non-infection in these months are probably related to the physiological condition of the host, which leads to the formation of a " gum-barrier " below the exposed surface ; this prevents the further progress of the fungus.

3. The ability of the spores of *S. purpureum* to infect woody tissues after varying periods of exposure has been tested throughout the year. It has been ascertained that it is very difficult for this fungus to infect exposures of a month's standing, and practically impossible for it to infect tissues which have been exposed for three months. *S. purpureum*

most readily infects woody tissues immediately after exposure. The causes of the failure to infect wounds after a certain lapse of time are discussed.

4. The bearing of these discoveries concerning the infectivity of *S. purpureum*, upon the practical treatment of the disease is discussed. It is urged that cutting out the dead wood or silvered branches (where the latter is advisable) and the thinning out of fruit trees should be done during the *early part of the summer.*

5. There seems to be no danger of *S. purpureum* following in the wake of an attack on plum trees by the fungi *Sclerotinia cinerea, Diaporthe perniciosa* and *Cytospora spp.*

6. The mode of formation of gum in response to wounding and to invasion by fungi, and the mode of development of protective " gum-barriers " is described.

7. It is pointed out that the amount of natural recovery from this disease is greater than was formerly supposed, even in susceptible varieties of plums such as Victoria and Czar. The amount of natural recovery has been correlated with the vigour of the tree. The immediate cause of natural recovery is the formation of a " gum-barrier " in the tissues which prevents the further growth of the fungus.

8. Experiments on the use of various protective substances for covering wounds in fruit trees are described. The best results have been obtained with soft grafting wax and with paints of a certain composition and consistency. The formulae for making these paints are given. Further research has shown that gas tar as well as Stockholm tar is an unsatisfactory protective.

9. The results of ring-barking silvered trees are recorded. As far as the results of the experiments are available they indicate that no considerable benefit is likely to accrue from this treatment.

10. The results of manuring silvered plum trees in various ways are given. Applications of basic slag and kainit have been found to benefit the trees in some instances, but it is too early to make a confident statement about the results of manurial treatment.

11. Treatment.

(*a*) Continued treatment of the disease along the lines of plant sanitation is advocated ; i.e., the fungus should be prevented from fructifying in and on the confines of fruit plantations.

(*b*) Cutting out of dead wood and living branches of fruit trees should be done in the early summer when the chances of infection by this

fungus are least. Broken branches should be cut out directly after the fruit is gathered.

(*c*) Fresh exposures of woody tissues should be covered immediately with soft grafting wax or with one of the paints described in the paper.

(*d*) The vigour of the trees should be maintained as high as possible by careful selection of land and young trees, and by suitable cultivation and manuring when the latter is necessary.

(*e*) A method is described by which health in silvered trees may be regained by stimulating the growth of branches arising lower down the tree than those badly affected by the disease.

12. It has been shown that the fungus in its growth secretes some substance which, apart from the living organism, will produce, when injected into healthy trees, some of the symptoms associated with the later phases of Silver-leaf disease.

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